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Transcriptional networks governing plant metabolism

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ABSTRACT

Efficiently obtaining and utilizing energy and elements is critical for an organism to maximize its fitness. Optimizing these processes requires precise regulation and coordination of an organism's metabolic networks in response to diverse environmental conditions and developmental stages. Metabolic regulation is often considered to largely occur by allosteric feedback where the metabolites directly influence the enzymes function. Recent work is showing that there is also an extensive role for transcriptional control of the enzyme encoding genes to construct the metabolic network in response to developmental and environmental stimuli. Within this review, we go through the extensive evidence of how transcription can coordinate the necessary metabolic shifts required to coordinate a plants metabolism with its development and environment. Additionally, we discuss evidence that the metabolites not only feed-back regulate the enzymes but also the upstream transcriptional processes, possibly to stabilize the system.

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1. Introduction

Efficiently obtaining and utilizing energy and elements is critical for an organism to maximize its fitness. Optimizing these processes requires precise regulation and coordination of an organism's metabolic networks in response to diverse environmental conditions and developmental stages [1,2]. These metabolic networks are the key avenues by which an organism obtains and produces all of the necessary building blocks for cells and the resulting biomass and they must be fine-tuned to make the most efficient use of resources available. This precise coordination is a foundational hypothesis for numerous fields of biology including predicting organismal growth and analyzing how a plant interacts with its environment [3]. This essential need to coordinate organismal metabolism has led to strong interest in understanding how metabolism is regulated [3,4].

A dominant feature of metabolic regulation is allosteric feedback whereby metabolites bind the enzymes within a biochemical pathway to control the activity of the enzymes and pathway flux [5,6]. This often leads to the argument that flux based measurements of metabolism are critical to understand metabolism and there is little reason to study the transcriptional control of metabolism [7–9]. This argument, however, is in stark contrast to the rapidly growing body of literature that shows that transcriptional control of metabolism is also a key component of plant metabolic regulation. One way we suggest to reconcile the transcriptional and allosteric views of metabolic regulation is that transcription may establish the base patterns of metabolism within cells in response to developmental and environmental cues [10–12] (Fig. 1). Flux regulation then works within the base rules that transcription establishes to precisely coordinate metabolic regulation. Within this review, we will summarize the evidence of the importance of metabolic transcriptional control by focusing on the intersection of metabolism with development and environmental stresses2. (Fig. 1). Then finally, we will discuss emerging evidence that metabolic outputs can feedback regulate the upstream transcriptional networks.

2. Metabolism and development are mutually dependent

Metabolism and metabolites are spatially organized across all levels of developmental organization for a breadth of reasons. A key example of metabolic spatial distribution is the presence of photosynthates in the shoots and their absence in roots that coordinates with the location of photosynthesis. Modern metabolomics platforms are greatly expanding our understanding of the precise developmental patterns displayed by metabolites by showing that they can be targeted to a diversity of different cell and tissue types in the plant [10,13–15]. In addition to metabolites being developmentally localized because of their function, the metabolites function

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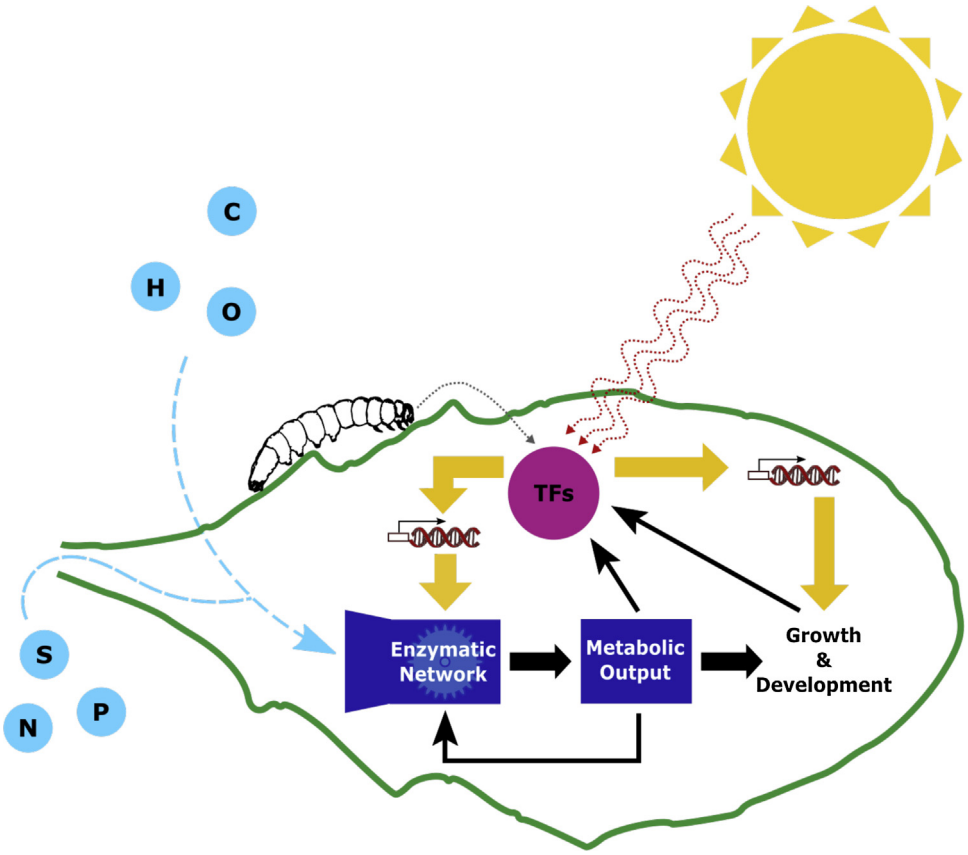

Fig. 1. A central role for TFs in plant metabolism. TFs differentially regulate expression of enzyme encoding genes by interacting with promoters to shape the potential enzymatic network as well as to control plant growth and development. The enzymatic network takes energy and chemical elements from the environment to generate the metabolites necessary for building plant cells, tissues and organs. The metabolites can modulate the enzymatic network via allosteric feedback. Additionally, TFs can coordinate metabolic shifts by integrating feedback signals from metabolic outputs and developmental processes, as well as integrating abiotic (example: UV-B radiation) and biotic stress (example: lepitdopteran herbivory) signals from the environment.

often plays a role in regulating specific developmental patterns. For example, auxin is transported and accumulates in very distinct domains in the root, and these auxin maxima specify meristematic regions and root cell identities [16]. Thus, regulation of auxin biosynthesis, transport and regulation must be strictly coordinated with the growth and development of the root, from cell type differentiation to cell elongation and maturation to ensure proper development of the plant. Essential to this coordination between development and metabolism are transcription factors (TFs) that regulate both developmental programs and metabolic pathways. To illustrate how recent research is discovering the coordination of development and metabolism, we will discuss three vignettes (i) Developmental TFs control metabolism essential to build the root vasculature, (ii) lipids accumulation in reproductive tissues and (iii) specialized defense metabolites localized in myrosin idioblasts. We were unable to describe all examples of the link between development and metabolism for spatial reasons.

2.1. Developmental TFs control metabolism essential to build the root vasculature

Central to the xylems' ability to transport water and nutrients from the roots to the aboveground parts of the plants are the fibers and lignin of the secondary cell walls that enable the formation of tracheid elements. The creation of these tracheid elements requires the precise timing of secondary cell wall deposition, lignification and finally programmed cell death. This requires proper timing of production of the requisite lignin monomers as any deviation in the pattern results in aberrant xylem vessels lacking structural integrity or subjected to embolisms later in developmental age. Thus, the formation of functional xylem requires the coordinated regulation of development and metabolism.

Recent work has begun to show how key TFs responsible for the initiation and development of xylem vessels also coordinate the production of metabolites required for secondary cell walls formation. The NAC domain transcription factors VASCULAR NAC DOMAIN 6 (VND6), VND7 and SECONDARY WALL ASSOCIATED NAC DOMAIN 1 (SND1) are key TFs that directly regulate xylem cell differentiation [17–20]. These TFs also regulate cellulose and lignin biosynthetic genes necessary for secondary cell wall deposition via the intermediary TFs MYB46 and MYB83 [21]. Overexpression of VND6, VND7 and MYB83 resulted in increased levels of secondary cell wall precursors and ectopic secondary cell wall formation, while knockout mutants of MYB46 and MYB83 displayed severe reduction of secondary cell wall thickening and loss of secondary cell wall precursors [17–21]. This hierarchical model of NAC to MYB to metabolic gene was recently changed when it was found that VND6 and VND7 can also directly interact with the promoters of enzyme encoding genes critical for the formation of metabolites required for secondary cell wall synthesis [22]. Thus, VND6 and VND7 create a regulatory feed-forward loop that likely helps to coordinate lignin metabolism with xylem development. Supporting this concept, when these VND6 and VND7 are mis-regulated, the coordination between root patterning in development and biosynthesis and delivery of metabolites is disrupted. Thus, the VND TFs play a role in bridging the initiation and final maturation of xylem vessel formation by linking regulatory control of development and the requisite metabolism.

2.2. TFs governing both morphogenesis and lipid metabolism programs in seed embryo development

Embryo development is divided into two temporally distinct phases of morphogenesis and maturation. Accumulation of lipids during the maturation phase provides energy stores for seed dormancy and germination when photosynthetic machinery is inactive. While lipid metabolism occurs on the whole plant level, lipid accumulation in the seeds is specifically controlled by several TFs involved in mid to late stages of embryogenesis and embryo maturation. Transcripts of LEAFY COTYLEDON (LEC1, LEC2 and FUS3), ABSCISIC ACID-INSENTIVE3 (ABI3) and WRINKLED1 (WRI1) TFs are found to overlap in the morphogenesis and maturation phase of embryo development, indicating their potential involvement in controlling embryo patterning and lipid accumulation in seeds [23–30].

LEC TFs and ABI3 are critical regulators of embryogenesis. Single and higher order mutants of these TFs resulted in early arrest of embryogenesis and switch to seedling development [25,26,28,29]. Ectopic expression of LEC TFs in vegetative tissues was sufficient to give rise to somatic embryos, demonstrating their roles in cell fate specification and totipotency [25,31]. Concurrently, while the LEC and ABI3 TFs regulate embryo development, they coordinate with each other to properly time lipid metabolism and accumulation in seeds after arrest in embryogenesis and at the beginning of the maturation phase. Studies measuring gene induction in response to LEC2 and WRI1 showed enzymes involved in fatty acid synthesis, including oleosin genes, are targets of these TFs [30,32,33]. In line with these findings, fatty acid analysis of *wri1* revealed decreases in total fatty acid accumulation in seeds and ectopic expression of LEC2 caused oil accumulation in leaves [24,33]. While LEC2 and WRI1 can regulate target lipid biosynthetic genes, LEC2 also creates a feed-forward loop wherein it also regulate lipid metabolism as an upstream regulator [24,33]. Thus, as with xylem formation, key developmental regulators also create feed-forward loops to directly regulate the necessary metabolic shifts in coordination with the development. While studies provided evidence of the dual roles these TFs play in both embryogenesis and lipid metabolism in seeds, the mechanism governing the switch between the two temporally distinct phases of seed embryo remain unclear [34,35].

2.3. Novel role of the conserved stomatal development TF, FAMA in specialized defense metabolism

In addition to primary metabolism, plant specialized defense metabolism also displays precise coordination of regulation to target specific metabolites and/or enzymes to explicit cells. For example, laticifers and glandular trichomes are unique repositories of defense metabolites in numerous species [36,37]. These defensive metabolites and their associated structures are highly evolutionarily labile with numerous independent events recreating similar developmental structures but it is not known how this independent evolution occurs [36,37]. Recent work on the development of myrosin idioblast (MI) fate and patterning is beginning to show how conserved TFs can be modified to evolve new tissues. MIs are specialized cells that contain myrosinase, an enzyme that activates defensive glucosinolates and in combination with other proteins turns them into toxic isothiocyanates, thiocyanates and nitriles to deter pathogens and herbivores [38–41]. Glucosinolates are unique to the Capparales and their accumulation varies in different plant organs as well as in different life cycle phases. The greatest accumulation of glucosinolates is found in seeds and lowest in leaves, though amounts of glucosinolate increase with leaf expansion [42,43]. The development of MIs is linked to the regulation of tissue- and organ-specific glucosinolate accumulation as a way for plants to strategize activating these toxic defense compounds without unnecessary and costly production of secondary metabolites.

In contrast to their importance for defense of *Arabidopsis* and other Capparales, it was not known how MI were initiated or patterned. Recent work has shown that the bHLH TF FAMA was required for the formation of the MI. Moreover, FAMA is required for the activation of myrosinase genes TRANSPARENT TESTA GALABRA 1 (*TGG1)* and *TGG2*, further evidence of FAMA's role in MI development [38]. Intriguingly, FAMA is a key regulator controlling cell division and cell differentiation in stomatal development, particularly specifying guard cell fate [38,44,45].

Moreover, FAMA and other bHLH TFs regulating stomatal development are conserved across seed plants [46]. This suggests that the specialized metabolism of glucosinolates in the Brassicaceae have co-opted the conserved function of FAMA to also function in regulating myrosin idioblast differentiation. This suggests the potential for a general pattern wherein new developmental structures are generated by co-opting conserved developmental TFs [36,37]. As with the NACs and LEC2, FAMA co-ordinately regulates both the developmental structure, MI, and the requisite enzyme, myrosinase. This raises the intriguing question of what stomatal metabolism might also be regulated by FAMA or if this metabolic function of FAMA is unique to the MIs [38,44,45].

2.4. Concluding remarks on transcriptional coordination of metabolism and development

Metabolites frequently intersect with developmental processes. While the TFs discussed in this section play a critical role in cell and tissue differentiation and regulating in the metabolism of those cells and tissues, they also highlight the beginning appearance of what may be a recurrent theme. In all instances, the developmental regulator also directly regulated the cell- or tissue-specific metabolism often via a feed-forward regulatory loop. Regulatory loops provide an enhanced ability to precisely coordinate different processes and as such may be a key feature of how TFs regulate both development and metabolism. This further suggests that as the two processes appear to be intrinsically linked, they are not mutually exclusive events and their study should be more coordinated in the future.

3. Stress and nutrient alteration of metabolism via transcription

In addition to development, metabolites are also central to the plants' response to a diverse array of environmental inputs. Examples include the accumulation of proline and polyamines in response to drought stress and altered lipid composition in response to freezing that are considered to be key changes facilitating the ability of the plant to survive these stresses [47,48]. These metabolic changes in response to stress are largely mediated via direct transcriptional shifts in the expression of enzyme-encoding genes that may enable shifts in the metabolic steady state to optimize growth within the new environmental or stressful condition. Recent work is beginning to highlight how TF networks modulate metabolism both to express key stress resistance metabolites and also to restructure the entire primary metabolic network to facilitate these shifts. We outline the transcriptional stress response to UV-B perception to illustrate a well-described regulatory pathway linking input to transcriptional control of metabolic output. In addition to stress regulation of metabolism, nutrient status is also a key component of metabolic regulation with plants having well characterized networks controlling the response to different carbon sources, nitrogen, phosphorous, iron, sulfur and a myriad of other nutrients. A full discussion of these pathways is beyond our

available space. Thus, we have decided to focus on nitrogen and sulfur to compare the considerably studied transcriptional control of nitrogen metabolism with the well modeled metabolic research but limited transcriptional studies of sulfur metabolism to provide an illustration of how combining these approaches is necessary for future research. Finally, we describe how these nutrient and environmental signals are being integrated as can be seen through the clock affecting both primary and secondary metabolism.

3.1. Direct pathway of UV-B photoperception to resistance metabolite expression

Phenylpropanoids, especially flavonoids, are critical metabolites to allow plants to live in the wild by absorbing ultraviolet-B radiation [49]. As a part of this function, the plant induces flavonoid biosynthesis in response to sensing UV-B [49,50]. Recent studies have developed the model of UV-B perception and the subsequent metabolic resistance response into what may be the best understood pathway linking environmental signal perception to transcriptional change in metabolic output to resist the stress (Fig. 2). UV-B is directly perceived by the UVR8 photoreceptor, which then transmits a signal to activate the TFs ELONGATED HYPOCOTYL 5 (HY5) and MYB12 [50–53]. The HY5 and MYB12 TFs create a feed-forward loop in which HY5 binds the MYB12 promoter and upregulates MYB12 and they both bind the promoters and upregulate the enzyme genes involved in flavonoid biosynthesis [54–57]. This transcriptional network then directly regulates the accumulation of the phenylpropanoids allowing for increased resistance to UV-B irradiation and survival in the wild [50,51,58]. This system provides a unique model whereby all of the direct molecular steps between the perception of a stress (UV-B/UVR8) and downstream transcriptional control of the metabolic enzyme genes to provide resistance to that stress are known. Interestingly, even in this largely linear pathway there are regulatory loops whose function in modulating the response remains to be determined (Fig. 2).

3.2. Remodulation of primary metabolism: nitrogen

A key component of any plant transcriptional response to stress is to properly control how the plant assimilates macronutrients and uses them in downstream reactions. For example, numerous studies have shown that many of the genes involved in the sensing, acquisition and downstream metabolic processes using nitrogen are transcriptionally responsive to diverse and stressful environmental conditions [59]. In accordance with this known transcriptional control for nitrogen metabolism, many studies have focused on discovering the involved TFs. These studies have identified at least a dozen TFs that control various aspects of nitrogen metabolism [60,61]. The best studied of these TFs is NIN-LIKE PROTEIN 7 (NLP7), which is retained in the nucleus quickly under nitrate sensing via an unknown signal. Once in the nucleus, NLP7 directly binds to the promoters of many core nitrogen responsive genes to regulate their expression [62]. NLP7 mutants are small and have a high shoot to root ratio, likely because the plants are suffering from limited nitrogen availability due to the altered nitrogen metabolic network [63]. However, most of the metabolic consequences of these nitrogen associated TFs are inferred based on the growth of mutant plants on different nutrients with minimal efforts to measure the broader impacts on metabolism, besides amino acid level, of these TF mutants. Thus, it is currently unknown how the transcriptional changes caused by these TFs actually affect the metabolic network and/or nitrogen metabolism within the plant. Developing this understanding of how TFs targeted to the primary metabolic network shift the actual metabolites in the network will be key to our understanding of the transcriptional link between stress and the resulting metabolic changes.

3.3. Remodulation of primary metabolism: sulfur

In contrast to the studies performed in regards to nitrogen metabolism, there have been extensive metabolic studies profiling and modeling sulfur metabolism that have been reviewed elsewhere [64]. The emphasis on modeling in this field has allowed researchers to predict accurately how changes in the plants genotype affect the accumulation of sulfurous metabolites [65]. In contrast, only a single transcription factor has been found to control transcription in response to altered sulfur status: SULFUR LIMITATION1 (SLIM1) also known as ETHYLENE INSENSITIVE LIKE 3 (EIL3). Mutant *slim1* plants suffer from severe limitations in sulfur availability and have a decrease in the content of sulfur-containing metabolite glutathione in their shoots [66]. No direct targets of SLIM1 have been identified; therefore its role in sulfur metabolism although clear, is linked indirectly to metabolic control. Incorporating the transcriptional control provided by genes like SLIM1 and other unidentified TFs into the extensive flux models has the potential to provide a more accurate model involving transcriptional and metabolic control of the system that could inform our understanding of how plants control their metabolic networks.

3.4. Integration of diverse stress transcriptional signals to coordinate metabolism: CCA1

Studies on the regulation of plant metabolism typically focused on the study of an individual pathway, environmental impact or nutrient source. While this singular focus has led to abundant knowledge about how individual metabolic pathways are regulated, genomic and systems biology tools are showing the importance of links across pathways and metabolites to coordinate responses [67–74].

An example of a gene that integrates information to coordinate the regulation of diverse metabolic networks is the phytochrome-activated, Myb-related TF CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) [75]. CCA1 potentially affects a majority of plant metabolism directly, as a TF, and indirectly, via its role as part of the circadian clock oscillator [73]. CCA1 directly binds the light-harvesting chlorophyll *a/b* protein (CAB1) promoter allowing it to regulate the expression of photosynthetic machinery [76]. Altered expression of CCA1 also leads to perturbation in the circadian rhythms that affect the expression of genes in the carbon cycle including starch accumulation, starch conversion to sugars, and downstream metabolic pathways that use the carbon backbones [77]. Metabolic profiling of the CCA1 overexpression line, however, showed that few metabolites had significantly altered abundance. This indicates that there are likely other regulatory factors that can counter misexpression of CCA1 to maintain the stability of central metabolism in arrhythmic growth [78].

In addition to controlling the carbon availability, CCA1 has been linked to mineral nutrient regulation. CCA1 directly targets the promoters and regulates the expression of the nitrogen metabolism genes glutamine synthase (GLN1;3) and glutamate dehydrogenase (GDH1) [79]. Moreover, CCA1 can indirectly affect the expression of nitrogen and sulfur assimilation via altered circadian regulation [80]. The CCA1 protein interacts with the HY5 protein and together can bind and regulate the promoters of CAB1 and CAB2 [81,82]. Therefore, CCA1 links together circadian regulation, nutrient metabolism, multiple light responses, and potentially pathways not yet analyzed. This ability of CCA1 to integrate diverse signals and influence numerous downstream pathways begins to illustrate how the hierarchical linear model of single pathway regulation

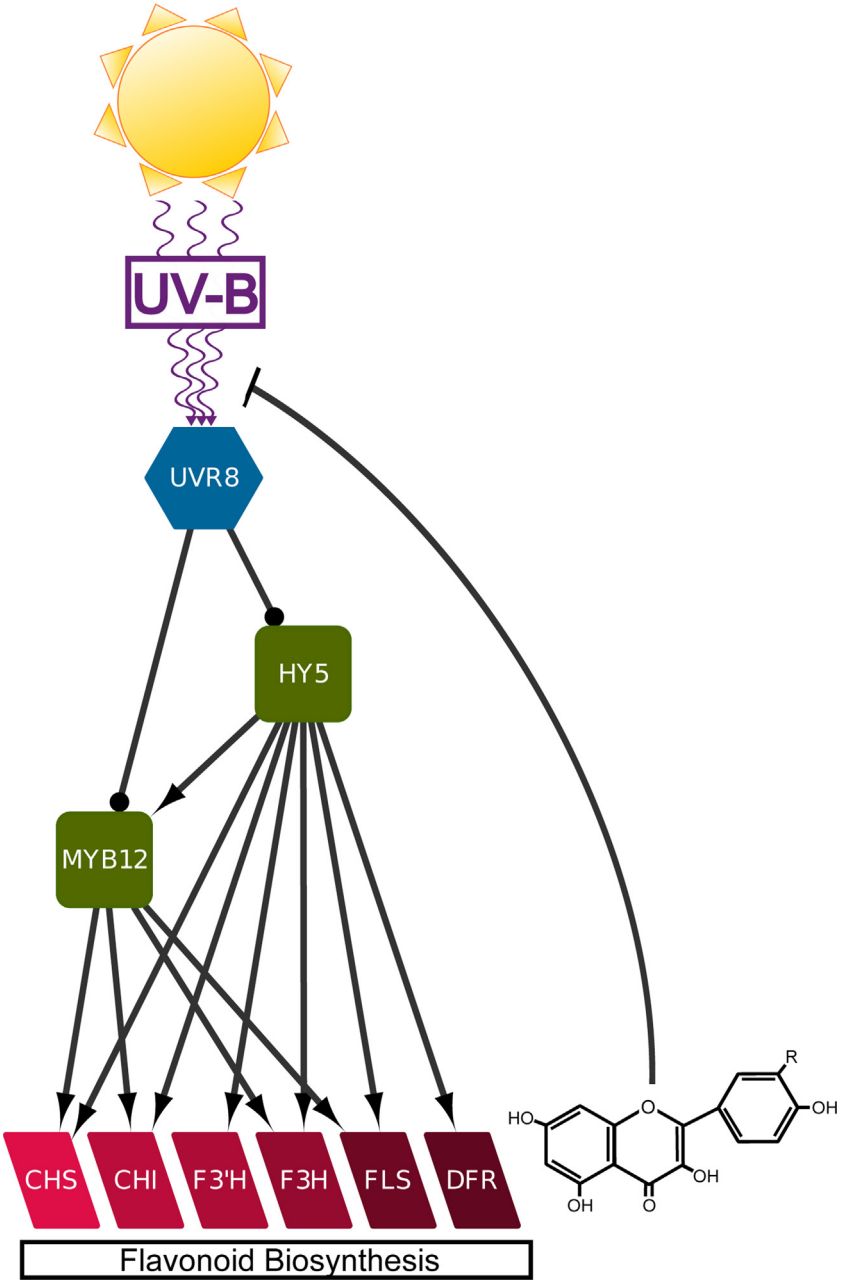

Fig. 2. Signalling pathway from UV-B perception to flavonoid biosynthesis. UV-B wavelengths from sunlight is directly perceived by the UVR8 photoreceptor (purple photons). UVR8 then induces HY5 and MYB12 expression (lines ending in circles). HY5 binds to the promoter of MYB12 and activates its expression creating a feed-forward transcriptional loop (line ending in arrow). HY5 and MYB12 bind to promoters of genes encoding enzymes in the flavonoid biosynthesis pathway (Rhombi) and activate their expression (lines ending in arrows). Finally, the flavonoids absorb the UV-B to prevent damage and possibly cause decreased UV-B photoperception.

breaks down when viewing the transcriptional regulatory mechanisms and their regulation of metabolic systems [69].

3.5. Concluding remarks on transcriptional coordination of metabolism and stress

Having a thorough understanding of the regulation of a metabolic system allows for prediction of how it will respond to a change in the state of the system. An altered state could be varying environmental conditions, a genetic perturbation, or an interaction of the two. Developing a predictive metabolic model will require combining flux models of metabolism with dynamic analysis of the transcriptional control of multiple metabolic pathways. Combining this knowledge is the next step to understand how these two levels of metabolism coordinate to change the plant's metabolic state.

4. Metabolites alter transcription to provide feedback and stabilize regulatory networks

The current model of transcriptional regulation within plants is largely based on the concept of a hierarchical regulatory system. This hierarchy posits that environmental signals stimulate a regulatory network that integrates these signals and then generates an output supposed to optimize the plants fitness. While this base model may include numerous circuits and interconnections, it is inherently linear because there are no connections between the output and the signaling network. However, systems engineer-

ing theory shows that these purely hierarchical/linear systems are highly unstable [83–85]. Stabilizing these hierarchical/linear systems requires the introduction of feedback whereby the outputs of the systems are linked to the inputs to allow the system to simultaneously coordinate both.

In the modeling of plant development, this link of output to input is directly built into the model by the inclusion of spatial constraints (i.e. tissue size and/or cell position) as a consequence of development [86,87]. Thus, the necessity of links between the output and input to stabilize these models has not always been widely recognized. In contrast to developmental models, models involving the transcriptional regulation of metabolic pathways or transcriptional responses do not allow for the inclusion of feedback by the metabolite or other outputs. This is in contrast to growing evidence that metabolites can provide this feedback regulation in plants and there is the potential that metabolite feedback of transcriptional pathways may be a ubiquitous albeit underappreciated facet of plant biology [70,88–90]. In this section, we will discuss several lines of evidence supporting this argument.

4.1. Metabolites and retrograde signaling

A key for any organism to modulate its metabolism is the ability to coordinate the functioning of organelles with the transcription of nuclear genes whose proteins are targeted to the organelles. This coordination involves retrograde signaling in which multiple signals from the organelle somehow migrate to the nucleus to control nuclear transcription [91–93]. Several pathways have been identified that enable retrograde signaling with all involving an organellar metabolite influencing the generation of the signal. The GUN (Genomes Uncoupled) pathway of retrograde signaling involves the likely detection of some metabolic component of the Heme biosynthetic pathway to affect nuclear expression of photosynthetic genes [93–95]. Another metabolite, methylerythritol cyclodiphosphate (MEcPP) derived from the plastid methylerythritol phosphate (MEP) pathway appears to conduct retrograde signaling by targeting a pool of stress responsive genes that largely focus on plant interactions with their biotic environment [96]. 3'-phosphoadenosine 5'-phosphate (PAP) also provides a retrograde signal that targets stress responsive nuclear genes largely linked to the abiotic environment such as drought and high light [97]. While these retrograde studies suggest that there is likely a breadth of metabolites that can influence gene transcription in plants, the underlying perception and signaling mechanisms have yet to be uncovered.

4.2. metabolic feedback loop involving phenylpropanoids and the Mediator transcriptional complex

Key evidence that metabolites and/or their direct consequences have the capacity to generate a feedback loop to regulate transcription and properly coordinate a regulatory network has come from the study of metabolic mutants affecting plant phenylpropanoid metabolism [70,98,99]. Mutating genes within the lignin/phenylpropanoid network frequently lead to plants with diminished growth, which had been presumed to be a side effect of the altered lignin [98]. This hypothesis has begun to be overturned by the application of a suppressor screen wherein *Arabidopsis ref8* mutants deficient in *p*-coumaroylshikimate 3′-hydroxylase (C3′H) was screened for second site mutations that rescued growth of this genotype. This screen found several second site suppressor mutations that alleviated the growth defect while maintaining the biochemical defect thus indicating that the biochemical deficiency was not causing the diminished growth. Cloning of these suppressors showed that they were mutated in components of Mediator, a multi-subunit complex that is required for eukaryotic transcription and influences numerous plant phenotypes [100]. were. The disruption of two Mediator subunits, MED5a/5b was able to rescue the growth phenotype and restore to near wild-type level of lignin albeit of a different biochemical form [70]. This suggests that the growth and lignin deficiencies were not a direct result of the biochemical mutation but instead caused by a regulatory effect triggered by the Mediator complex in response to the biochemical mutation and the plants altered metabolism [70].

This hypothesis was supported in a separate suppressor screen of another phenylpropanoid mutant, *fah1*, that had an unexplained repressed accumulation of hydroxycinnamate ester (HCE) and anthocyanins. Mutations in the MED5a/5b genes within this *fah1* mutant lead to a recovered ability to synthesis and accumulate HCEs and anthocyanins [99]. Thus, the decreased accumulation of HCEs and anthocyanins was not due to the biochemical deficiency in the fah1 enzyme but was instead caused by a regulatory response that works via Mediator and is triggered by the fah1 mutation. A similar link between Mediator based regulation of the phenylpropanoid pathway and biosynthetic deficiency was found using a mutant in the indole glucosinolate biosynthesis pathway that altered phenylpropanoid biosynthesis [101]. Similarly, mutations in Mediator again recovered the proper expression of the phenylpropanoid pathway suggesting that Mediator is not just involved in regulating the phenylpropanoid pathway in response to phenylpropanoid mutations but also for other biochemical deficiencies. However, in all cases, the immediate signal stimulating this transcriptional effect remains to be identified.

In agreement with the concept that the above three examples outlined may be examples of metabolite-facilitated feedback, the Mediator complex is a direct regulator of transcription for the phenylpropanoid genes [71]. Thus, the Mediator complex regulates the phenylpropanoid pathway and is sensitive to perturbations in the output of this complex establishing a feedback loop whereby the output can influence the input. Interestingly, this link between lignin and anthocyanin metabolism and transcriptional regulation is not the only observed link for phenlypropanoids. There has also been a link between flavonoid flux and potential regulatory consequences independent of the above system [88]. Thus, it is likely that multiple aspects of the phenylpropanoid biosynthetic pathways can influence the plant transcriptional networks.

4.3. Rapid evolution of metabolic signals

In addition to the above molecularly characterized examples where conserved primary metabolites cause what appear to be conserved transcriptional signaling events, there is a rapid growth in the observation of plant secondary metabolites also having potential transcriptional regulatory effects upon the plant. In *Arabidopsis thaliana*, there is evidence that an indolic glucosinolate defense compound can lead to altered defense responses like altered callose formation [102]. Interestingly, DIMBOA, a monocot limited indolic secondary metabolite, has also been linked to altered regulation of callose formation in plant/pathogen interactions in maize [103]. Thus, two separate plant lineages have evolved a regulatory link between evolutionarily distinct indolic metabolites and callose formation [102,103]. One possible solution to how divergent metabolites can lead to conserved transcriptional responses was recently suggested by the possibility that regulatory proteins that perceive plant defense compounds can coevolve with the structural variation in distinct plant lineages [104]. This allows the metabolites structure to change while maintaining the same regulatory linkage.

There have also been examples of plant secondary metabolite having regulatory influence over non-defense mechanisms. In *Raphanus sativus,* a glucosinolate hydrolysis product, raphanusanin, can directly modulate plant growth by interacting with the

TIR1 auxin receptor to alter the downstream transcription of auxin responsive genes and the resulting hypocotyl response [105,106]. In *Arabidopsis thaliana*, variation in the glucosinolate structure has been linked to altered circadian clock transcriptional patterns [89]. In oat, beta-amyrin has been associated with altered cell patterning in the roots [107]. While the molecular mechanism of most of these regulatory connections remains to be determined, there is a recurrent pattern where plant metabolites have the ability to provide feedback linkages to broad aspects of plant physiology that are under transcriptional control. Only by cloning all of the underlying genes for a number of these different connections will we be able to understand how these connections have developed and how many other metabolites may have similar but unmeasured links.

5. Conclusions

As shown above, recent work is beginning to highlight the role of transcription factors in controlling plant metabolism to potentially optimize growth under diverse conditions and tissues. A common feature of all these studies is the frequent observation of regulatory loops. These can be either feed-forward loops involving TFs regulating each other and a common enzyme-encoding gene (i.e. LEC2/WRI1/Lipids) or feedback loops wherein the metabolic output of a network influences the activity of TFs within the plant (i.e. phenylpropanoids/Mediator). This leads us to propose that the largely hierarchical modeling of plant metabolic regulation is insufficient to the task at hand and instead we need to move into alternative approaches that can better integrate these regulatory loops. Similarly, this suggests that plant metabolism, due to its ease of manipulation and measurement, may provide the optimal platform to conduct these integrative studies on linking TFs to their downstream consequences in transcription, metabolism and resulting growth phenotypes. It will be exciting to see the outcomes of these future integrative systems experiments studying how TFs and metabolic flux interconnect in plants.

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References

[1] U. Sauer, D.R. Lasko, J. Fiaux, M. Hochuli, R. Glaser, et al., Metabolic Flux Ratio Analysis of Genetic and Environmental Modulations of *E. coli* Central Carbon, Metabol. J. Bacteriol. 181 (1999) 6679–6688.
[2] P.D. Keightley, Models of quantitative variation of flux in metabolic pathways, Genetics 121 (1989) 869–876.
[3] Karban, R., Baldwin, I.T. 1997. Induced Responses to Herbivory. Chicago, IL, USA. University of Chicago Press.
[4] A.M. Smith, M. Stitt, Coordination of carbon supply and plant growth, Plant Cell Environ. 30 (2007) 1126–1149.
[5] J. Li, R.L. Last, The *Arabidopsis thaliana* trp5 mutant has a feedback-resistant anthranilate synthase and elevated soluble tryptophan, Plant Physiol. 110 (1996) 51–59.
[6] V.-R. Chellamuthu, E. Ermilova, T. Lapina, J. Lueddecke, E. Minaeva, et al., A widespread glutamine-sensing mechanism in the plant kingdom, Cell 159 (2014) 1188–1199.
[7] Y. Shachar-Hill, Metabolic network flux analysis for engineering plant systems, Curr. Opin. Biotechnol. 24 (2013) 247–255.
[8] D.K. Allen, I.G.L. Libourel, Y. Shachar-Hill, Metabolic flux analysis in plants: coping with complexity, Plant Cell Environ. 32 (2009) 1241–1257.
[9] I.G.L. Libourel, Y. Shachar-Hill, Metabolic flux analysis in plants: from intelligent design to rational engineering, Ann. Rev. Plant Biol. (2008) 625–650.
[10] A. Moussaieff, I. Rogachev, L. Brodsky, S. Malitsky, T.W. Toal, et al., High-resolution metabolic mapping of cell types in plant roots, Proc. Natl. Acad. Sci. U. S. A 110 (2013) E1232–E1241.
[11] J.R. Dinneny, T.A. Long, J.Y. Wang, J.W. Jung, D. Mace, et al., Cell identity mediates the response of *Arabidopsis* roots to abiotic stress, Science 320 (2008) 942–945.
[12] S.M. Brady, D.A. Orlando, J.Y. Lee, J.Y. Wang, J. Koch, et al., A high-resolution root spatiotemporal map reveals dominant expression patterns, Science 318 (2007) 801–806.
[13] R. Shroff, F. Vergara, A. Muck, A. Svatos, J. Gershenzon, Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense, Proc. NatL. Acad. Sci. U. S. A. 105 (2008) 6196–6201.
[14] A. Maruyama-Nakashita, Y. Nakamura, T. Tohge, K. Saito, H. Takahashi, *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism, Plant Cell 18 (2006) 3235–3251.
[15] S. Krueger, P. Giavalisco, L. Krall, M.C. Steinhauser, D. Bussis, et al., A Topological map of the compartmentalized *Arabidopsis thaliana* leaf metabolome, Plos One 6 (2011).
[16] S. Sabatini, D. Beis, H. Wolkenfelt, J. Murfett, T. Guilfoyle, et al., An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root, Cell (1999) 463–472.
[17] K. Ohashi-Ito, Y. Oda, H. Fukuda, *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed death and secondary wall formation during xylem differentiation *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed death and secondary wall formation during xylem differentiation, Plant Cell (2010) 3461–3473.
[18] M. Yamaguchi, N. Goue, H. Igarashi, M. Ohtani, Y. Nakano, et al., VASCULAR-RELATED NAC-DOMAIN6 and VASCULAR-RELATED NAC-DOMAIN7 effectively induce transdifferentiation into xylem vessel elements under control of an induction system, Plant Physiol. 153 (2010) 906–914.
[19] M. Yamaguchi, M. Kubo, H. Fukuda, T. Demura, VASCULAR-RELATED NAC-DOMAIN7 is Involved in the Differentiation of All Types of Xylem Vessels in *Arabidopsis* Roots and Shoots, Plant J: Blackwell Publishing Ltd., 2008, pp. 652–664.
[20] M. Yamaguchi, N. Mitsuda, M. Ohtani, M. Ohme-Takagi, K. Kato, et al., VASCULAR-RELATED NAC-DOMAIN 7 Directly Regulates the Expression of a Broad Range of Genes for Xylem Vessel Formation, Plant J: Blackwell Publishing Ltd., 2011, pp. 579–590.
[21] R.L. McCarthy, R. Zhong, Z.-H. Ye, MYB83 Is a Direct Target of SND1 and acts redundantly with myb 46 in the regulation of secondary cell wall biosynthesis in *Arabidopsis*, Plant Cell Physiol (2009) 1950–1964, Oxford University Press.
[22] M. Taylor-Teeples, L. Lin, M. de Lucas, G. Turco, T.W. Toal, et al., An *Arabidopsis* gene regulatory network for secondary cell wall synthesis, Nature (2014), Nature: Publishing Group.
[23] J.G. Angeles-Núñez, A. Tiessen, Mutation of the transcription factor LEAFY COTYLEDON 2 alters the chemical composition of *Arabidopsis* seeds, decreasing oil and protein content, while maintaining high levels of starch and sucrose in mature seeds, J. Plant Physiol. (2011) 1891–1900.
[24] S. Baud, M.S. Mendoza, A. To, E. Harscoët, L. Lepiniec, et al., WRINKLED1 specifies the regulatory action of LEAFY COTYLEDON2 towards fatty acid metabolism during seed maturation in *Arabidopsis*, Plant J. (2007) 825–838.
[25] J.J. Harada, Role of *Arabidopsis* LEAFY COTYLEDON genes in seed development, J. Plant Physiol. (2001).
[26] F. Parcy, C. Valon, A. Kohara, S. Miséra, J. Giraudat, The ABSCISIC ACID-INSENSITIVE3, FUSCA3, and LEAFY COTYLEDON1 loci act in concert to control multiple aspects of *Arabidopsis* seed development, Plant Cell: Am. Soc. Plant Biol. (1997) 1265–1277.
[27] M. Santos-Mendoza, B. Dubreucq, M. Miquel, M. Caboche, M.L. Lepiniec, LEAFY COTYLEDON 2 activation is sufficient to trigger the accumulation of oil and seed specific mRNAs in *Arabidopsis* leaves, FEBS Lett. (2005) 4666–4670.
[28] S.L. Stone, S.A. Braybrook, S.L. Paula, L.W. Kwong, J. Meuser, et al., Arabidopsis LEAFY COTYLEDON2 induces maturation traits and auxin activity: implications for somatic embryogenesis, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 3151–3156.
[29] M. West, K.M. Yee, J. Danao, J.L. Zimmerman, R.L. Fischer, et al., LEAFY COTYLEDON1 Is an Essential Regulator of Late Embryogenesis and Cotyledon Identity in *Arabidopsis*, Plant Cell, Am. Soc. Plant Biol. (1994) 1731–1745.
[30] K. Maeo, T. Tokuda, A. Ayame, N. Mitsui, T. Kawai, et al., An AP2-type transcription factor, WRINKLED1, of *Arabidopsis thaliana* binds to the AW-box sequence conserved among proximal upstream regions of genes involved in fatty acid synthesis, Plant J. (2009) 476–487, Blackwell Publishing Ltd.
[31] S.L. Stone, L.W. Kwong, K.M. Yee, J. Pelletier, L. Lepiniec, et al., LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 11806–11811.
[32] S.A. Braybrook, S.L. Stone, S. Park, A.Q. Bui, B.H. Le, et al., Genes directly regulated by LEAFY COTYLEDON2 provide insight into the control of embryo maturation and somatic embryogenesis, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 3468–3473.

[33] A. To, J. Joubès, G. Barthole, A. Lécureuil, A. Scagnelli, et al., WRINKLED transcription factors orchestrate tissue-specific regulation of fatty acid biosynthesis in *Arabidopsis*, The Plant Cell (2012) 5007–5023, American Society of Plant Biologists.
[34] C.S. Johnson, B. Kolevski, D.R. Smyth, TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of *Arabidopsis* encodes a WRKY transcription factor, Plant Cell (2002) 1359–1375.
[35] T. Ishida, S. Hattori, R. Sano, K. Inoue, Y. Shirano, et al., *Arabidopsis* TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation *Arabidopsis* TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation, Plant Cell (2007) 2531–2543.
[36] D.J. Kliebenstein, New synthesis-regulatory evolution, the veiled world of chemical diversification, J. Chem. Ecol. 39 (2013) 349.
[37] Kliebenstein D.J. 2013. Making new molecules — evolution of structures for novel metabolites in plants. Curr Opin Plant Biol Online.
[38] M. Li, F.D. Sack, Myrosin idioblast cell fate and development are regulated by the arabidopsis transcription factor FAMA the auxin pathway, and vesicular trafficking, Plant Cell Am. Soc. Plant Biol. (2014) 4053–4066.
[39] M. Burow, A. Losansky, R. Muller, A. Plock, D.J. Kliebenstein, et al., The genetic basis of constitutive and herbivore-induced ESP-independent nitrile formation in *Arabidopsis*, Plant Physiol. 149 (2009) 561–574.
[40] M. Burow, Z.Y. Zhang, J.A. Ober, V.M. Lambrix, U. Wittstock, et al., ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in *Arabidopsis*, Phytochemistry 69 (2008) 663–671.
[41] M. Burow, M. Rice, B. Hause, U. Wittstock, J. Gershenzon, Cell- and tissue-specific localization and regulation of the epithiospecifier protein in *Arabidopsis thaliana*, Plant Mol.Biol. 64 (2007) 173–185.
[42] P.D. Brown, J.G. Tokuhisa, M. Reichelt, J. Gershenzon, Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*, Phytochem 62 (2003) 471–781.
[43] I.E. Sønderby, F. Geu-Flores, B.A. Halkier, Biosynthesis of glucosinolates — gene discovery and beyond, Trends Plant Sci. 15 (2010) 283–290.
[44] D.C. Bergmann, W. Lukowitz, C.R. Somerville, Stomatal development and pattern controlled by a MAPKK kinase, Sci.: Am. Assoc. Adv. Sci. (2004) 1494–1497.
[45] K. Ohashi-Ito, D.C. Bergmann, *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development, Plant Cell: Am. Soc. Plant Biol. (2006) 2493–2505.
[46] C.A. MacAlister, D.C. Bergmann, Sequence and function of basic helix-loop-helix proteins required for stomatal development in *Arabidopsis* are deeply conserved in land plants, Evol. Dev. (2011) 182–192.
[47] M. Seki, T. Umezawa, K. Urano, K. Shinozaki, Regulatory metabolic networks in drought stress responses, Curr. Opin. Plant. Biol. 10 (2007) 296–302.
[48] R. Welti, W. Li, M. Li, Y. Sang, H. Biesiada, et al., Profiling membrane lipids in plant stress responses Role of phospholipase D-alpha in freezing-induced lipid changes in *Arabidopsis*, J. Biol. Chem. 277 (31) (2002) 994–32002.
[49] L.G. Landry, C.C.S. Chapple, R.L. Last, *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced Ultraviolet-B injury and oxidative damage *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced Ultraviolet-B injury and oxidative damage, Plant Physiol. 109 (1995) 1159–1166.
[50] D.J. Kliebenstein, J.E. Lim, L.G. Landry, R.L. Last, *Arabidopsis* UVR8 regulates ultraviolet-B signal transduction and tolerance and contains sequence similarity to human Regulator of Chromatin Condensation 1 *Arabidopsis* UVR8 regulates ultraviolet-B signal transduction and tolerance and contains sequence similarity to human Regulator of Chromatin Condensation 1, Plant Physiol. 130 (2002) 234–243.
[51] B.A. Brown, C. Cloix, G.H. Jiang, E. Kaiserli, P. Herzyk, et al., A UV-B-specific signaling component orchestrates plant UV protection, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 18225–18230.
[52] C. Cloix, G.I. Jenkins, Interaction of the *Arabidopsis* UV-B-specific signaling component UVR8 with chromatin, Mol. Plant 1 (2008) 118–128.
[53] C. Cloix, G.I. Jenkins, Interaction of the *Arabidopsis* UV-B-specific signaling component UVR8 with chromatin, Mol. Plant 1 (2008) 118–128.
[54] F. Mehrtens, H. Kranz, P. Bednarek, B. Weisshaar, The *Arabidopsis* transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis, Plant Physiol. 138 (2005) 1083–1096.
[55] R. Stracke, J.J. Favory, H. Gruber, L. Bartelniewoehner, S. Bartels, et al., The *Arabidopsis* bzip transcription factor hy5 regulates expression of the PFG1/MYB12 gene in response to light and ultraviolet-b radiation, Plant Cell Environ. 33 (2010) 88–103.
[56] BINKERTM, L. Kozma-Bognár, K. Terecskei, L. De Veylder, F. Nagy, et al., UV-B-responsive association of the *Arabidopsis* bZIP transcription factor ELONGATED HYPOCOTYL5 with target genes, including its own promoter, Plant Cell 26 (2014) 4200–4213.
[57] T. Tohge, M. Kusano, A. Fukushima, K. Saito, A.R. Fernie, Transcriptional and metabolic programs following exposure of plants to UV-B irradiation, Plant Signal. Behav. 6 (2011) 1987–1992.
[58] H. Jin, E. Cominelli, P. Bailey, A. Parr, F. Mehrtens, et al., Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*, EMBO J. 19 (2000) 6150–6161.
[59] N.M. Crawford, A.D.M. Glass, Molecular and physiological aspects of nitrate uptake in plants, Trends Plant Sci. 3 (1998) 389–395.
[60] R.A. Gutierrez, Systems biology for enhanced plant nitrogen nutrition, Science 336 (2012) 1673–1675.
[61] P. Guan, R. Wang, P. Nacry, G. Breton, S.A. Kay, et al., Nitrate foraging by *Arabidopsis* roots is mediated by the transcription factor TCP 20 through the systemic signaling pathway, Proc. Natl. Acad. Sci. U. S. A. (2014).
[62] C. Marchive, F. Roudier, L. Castaings, V. Brehaut, E. Blondet, et al., Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants, Nat. Commun. 4 (2013) 1713.
[63] L. Castaings, A. Camargo, D. Pocholle, V. Gaudon, Y. Texier, et al., The nodule inception-like protein 7 modulates nitrate sensing and metabolism in *Arabidopsis*, Plant J. 57 (2009) 426–435.
[64] A. Calderwood, R.J. Morris, S. Kopriva, Predictive sulfur metabolism — a field in flux, Frontiers in Plant Science 5 646 (2014).
[65] G. Curien, S. Ravanel, R. Dumas, A kinetic model of the branch-point between the methionine and threonine biosynthesis pathways in *Arabidopsis* thaliana, Eur. J. Biochem. 270 (2003) 4615–4627.
[66] A. Maruyama-Nakashita, Y. Nakamura, T. Tohge, K. Saito, H. Takahashi, *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism, Plant Cell Online 18 (2006) 3235–3251.
[67] B. Li, D.J. Kliebenstein, The AT-hook Motif-encoding Gene METABOLIC NETWORK MODULATOR 1 underlies natural variation in *Arabidopsis* primary metabolism, Front. Plant Sci. 5 (2014).
[68] M. Taylor-Teeples, L. Lin, M. de Lucas, G. Turco, C. Doherty, et al., Environmental developmental and genotype-dependent regulation of xylem cell specification and secondary cell wall biosynthesis in *Arabidopsis thaliana*, Nature Accepted (2014).
[69] B. Li, A. Gaudinier, M. Taylor-Teeples, N.T. Nham, C. Ghaffari, et al., Promoter based integration in plant defense regulation, Plant Physiol. 166 (2014) 1803–1820.
[70] N.D. Bonawitz, J.I. Kim, Y. Tobimatsu, P.N. Ciesielski, N.A. Anderson, et al., Disruption of mediator rescues the stunted growth of a lignin-deficient *Arabidopsis mutant*, Nature 509 (2014) 376–380.
[71] N.D. Bonawitz, W.L. Soltau, M.R. Blatchley, B.L. Powers, A.K. Hurlock, et al., REF4 and RFR1, subunits of the transcriptional coregulatory complex mediator, are required for phenylpropanoid homeostasis in *Arabidopsis*, J. Biol. Chem. 287 (2012) 5434–5445.
[72] M.F. Covington, J.N. Maloof, M. Straume, S.A. Kay, S.L. Harmer, Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development, Genome Biol. 9 (2008) R130.
[73] S.L. Harmer, The circadian system in higher plants, Ann. Rev. Plant Biol. 60 (2009) 357–377.
[74] S.L. Harmer, S.A. Kay, Positive and negative factors confer phase-specific circadian regulation of transcription in *Arabidopsis*, Plant Cell 17 (2005) 1926–1940.
[75] Z.Y. Wang, E.M. Tobin, Constitutive Expression of the CIRCADIAN CLOCK ASSOCIATED 1(CCA1) gene disrupts circadian rhythms and suppresses Its Own . . ., Cell 93 (1998) 1207–1217.
[76] Z.Y. Wang, D. Kenigsbuch, L. Sun, E. Harel, M.S. Ong, et al., A Myb-related transcription factor is involved in the phytochrome regulation of an *Arabidopsis Lhcb* gene, Plant Cell Online 9 (1997) 491–507.
[77] S.M. Smith, D.C. Fulton, T. Chia, D. Thorneycroft, A. Chapple, et al., Diurnal changes in the transcriptome encoding enzymes of starch metabolism provide evidence for both transcriptional and posttranscriptional regulation of starch metabolism in *Arabidopsis* leaves, Plant Physiol. 136 (2004) 2687–2699.
[78] A. Fukushima, M. Kusano, N. Nakamichi, M. Kobayashi, N. Hayashi, et al., Impact of clock-associated *Arabidopsis* pseudo-response regulators in metabolic coordination, Proc. Natl. Acad. Sci. 106 (2009) 7251–7256.
[79] R.A. Gutierrez, T.L. Stokes, K. Thum, X. Xu, M. Obertello, et al., Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene CCA1, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 4939–4944.
[80] S.L. Harmer, Orchestrated transcription of key pathways in *Arabidopsis* by the circadian Clock, Science 290 (2000) 2110–2113.
[81] S. Chattopadhyay, L.H. Ang, P. Puente, X.W. Deng, N. Wei, *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression, Plant Cell Online 10 (1998) 673–683.
[82] C. Andronis, S. Barak, S.M. Knowles, S. Sugano, E.M. Tobin, The clock protein CCA1 and the bZIP transcription factor HY5 physically interact to regulate gene expression in *Arabidopsis*, Mol. Plant 1 (2008) 58–67.
[83] E.J. Chikofsky, J.H. Cross, Reverse engineering and design recovery — A taxonomy, Ieee Software 7 (1990) 13–17.
[84] I. Dobson, B.A. Carreras, V.E. Lynch, D.E. Newman, Complex systems analysis of series of blackouts: Cascading failure, critical points, and self-organization, Chaos 17 (2007).
[85] E. Eilam, Reversing: Secrets of Reverse Engineering, Wiley Publishing, 595, 2005.
[86] G.D. Bilsborough, A. Runions, M. Barkoulas, H.W. Jenkins, A. Hasson, et al., Model for the regulation of *Arabidopsis thaliana* leaf margin development, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 3424–3429.

[87] O. Hamant, M.G. Heisler, H. Jonsson, P. Krupinski, M. Uyttewaal, et al., Developmental Patterning by Mechanical Signals in *Arabidopsis*, Science 322 (2008) 1650–1655.
[88] L. Pourcel, N.G. Irani, A.J.K. Koo, A. Bohorquez-Restrepo, G.A. Howe, et al., A chemical complementation approach reveals genes and interactions of flavonoids with other pathways, Plant J 74 (2013) 383–397.
[89] R.E. Kerwin, J.M. Jiménez-Gómez, D. Fulop, S.L. Harmer, J.N. Maloof, et al., Network quantitative trait loci mapping of circadian clock outputs identifies metabolic pathway-to-clock linkages in *Arabidopsis*, Plant Cell 23 (2011) 471–485.
[90] W.L. Araujo, A. Nunes-Nesi, Z. Nikoloski, L.J. Sweetlove, A.R. Fernie, Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues, Plant Cell Environ. 35 (2012) 1–21.
[91] A. Nott, H.-S. Jung, S. Koussevitzky, J. Chory, Plastid-to-nucleus retrograde signaling, Ann. Rev. Plant Biol. (2006) 739–759.
[92] S. Koussevitzky, A. Nott, T.C. Mockler, F. Hong, G. Sachetto-Martins, et al., Signals from chloroplasts converge to regulate nuclear gene expression, Science 316 (2007) 715–719.
[93] J.D. Woodson, J. Chory, Coordination of gene expression between organellar and nuclear genomes, Nature Rev. Gen. 9 (2008) 383–395.
[94] G. Vinti, A. Hills, S. Campbell, J.R. Bowyer, N. Mochizuki, et al., Interactions between hy1 and gun mutants of *Arabidopsis*, and their implications for plastid/nuclear signalling, Plant J. 24 (2000) 883–894.
[95] R.M. Larkin, J.M. Alonso, J.R. Ecker, J. Chory, GUN4, a regulator of chlorophyll synthesis and intracellular signaling, Science 299 (2003) 902–906.
[96] Y. Xiao, T. Savchenko, E.E.K. Baidoo, W.E. Chehab, D.M. Hayden, et al., Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes, Cell 149 (2012) 1525–1535.
[97] G.M. Estavillo, P.A. Crisp, W. Pornsiriwong, M. Wirtz, D. Collinge, et al., Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*, Plant Cell 23 (2011) 3992–4012.
[98] J.I. Kim, P.N. Ciesielski, B.S. Donohoe, C. Chapple, X. Li, Chemically induced conditional rescue of the reduced epidermal fluorescence8 mutant of *Arabidopsis* reveals rapid restoration of growth and selective turnover of secondary metabolite pools, Plant Physiol. 164 (2014) 584–595.
[99] N. Anderson, N.D. Bonawitz, K.E. Nyffeler, C. Chapple, Loss of ferulate 5-hydroxylase leads to Mediator-dependent inhibition of soluble phenylpropanoid biosynthesis in *Arabidopsis*, Plant Physiol. (2015).
[100] R.D. Kornberg, The molecular basis of eukaryotic transcription, Proc. Natl. Acad. Sci. 104 (2007) 12955–12961.
[101] J.I. Kim, W.L. Dolan, N.A. Anderson, C. Chapple, Indole glucosinolate biosynthesis limits phenylpropanoid accumulation in *Arabidopsis thaliana*, Plant Cell 27 (2015) 1529–1546.
[102] N.K. Clay, A.M. Adio, C. Denoux, G. Jander, F.M. Ausubel, Glucosinolate metabolites required for an *Arabidopsis* innate immune response, Science 323 (2009) 95–101.
[103] L.N. Meihls, V. Handrick, G. Glauser, H. Barbier, H. Kaur, et al., Natural variation in maize aphid resistance is associated with 2,4-Dihydroxy-7-Methoxy-1,4-Benzoxazin-3-One glucoside methyltransferase activity, Plant Cell 25 (2013) 2341–2355.
[104] Heinze, M., Brandt, W., Marillonnet, S., Roos, W., 2015, Self and Non-Self in the Control of Phytoalexin Biosynthesis: Plant Phospholipases A2 with Alkaloid-Specific Molecular Fingerprints. In Press.
[105] T. Hasegawa, K. Yamada, S. Kosemura, S. Yamamura, K. Hasegawa, Phototropic stimulation induces the conversion of glucosinolate to phototropism-regulating substances of radish hypocotyls, Phytochemistry 54 (2000) 275–279.
[106] K. Yamada, T. Hasegawa, E. Minami, N. Shibuya, S. Kosemura, et al., Induction of myrosinase gene expression and myrosinase activity in radish hypocotyls by phototropic stimulation, J, Plant Physiol 160 (2003) 255–259.
[107] A.C. Kemen, S. Honkanen, R.E. Melton, K.C. Findlay, S.T. Mugford, et al., Investigation of triterpene synthesis and regulation in oats reveals a role for beta-amyrin in determining root epidermal cell patterning, Proc, Nat, Acad, Sci. U. S. A. 111 (2014) 8679–8684.